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"SAFE AND EFFECTIVE STIMULATION OF NEURAL TISSUE"

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ABSTRACT AND INTRODUCTION

During the past quarter we have conducted physiologic and histologic evaluations of two types of microstimulation protocols in the cat's sensorimotor cortex. The first is a protocol which has been used to produce stable phosphenes in the human visual cortex (Schmidt et al., 1996), as well as variants of that protocol in which the duty cycle was increased. The second evaluation was an effort to determine the neural damage threshold when arrays of microelectrodes are pulsed at high stimulus intensities. These studies indicate that the visual prosthesis protocol used by Schmidt, et al., or the variants of that protocol, does not induce morphologically detectable neuronal damage. Although further work is indicated, the neural damage threshold for microstimulation of the cat's sensorimotor cortex appears to be between 20 nC/ph (1,000 μC/cm²) and 40 nC/ph (2,000 μC/cm²) during continuous stimulation at 50 Hz for 7 hours.

METHODS

Fabrication of the microelectrode arrays. The shafts of the discrete iridium microelectrodes are made from iridium wire, 35 μm in diameter. One end of each shaft is etched electrolytically to a cone with an included angle of 10° and with a blunt hemispheric tip approximately 12 μm in diameter. After the tips have been shaped to the proper configuration, a Teflon-insulated wire lead is micro-welded near the upper end of the shaft. The shaft is then insulated with 4 thin coats of Epoxylite electrode varnish, and each layer of insulation is baked using a schedule recommended by the manufacturer. The insulation is removed from the tip of the shafts by either an erbium or (recently) an excimer laser. The surface area of the exposed tip is determined by measurement of the double-layer capacitance while the tip is immersed in phosphate-buffered saline solution, using fast (100 Hz) cyclic voltammetry. The surface areas of these electrodes was 2,000 ± 400 μm². The individual microelectrodes are then assembled into arrays of 16, which extend 1.1 to 1.2 mm from an epoxy matrix, which is 3 mm in diameter. In addition, the arrays contain 3 electrically inactive stabilizing pins, whose tracks also serve as fiducial marks for identifying the individual electrode during the subsequent histologic analysis. The microelectrodes are then "activated" (a layer of

high-valence iridium oxide formed by anodic conversion) by potentiodynamic cycling between -0.8 and +0.7 volts with respect to a saturated calomel electrode, with the microelectrodes immersed in saturated sodium phosphate solution. The activation process is terminated when each microelectrode has a total charge capacity of 20 nC.

Cleaning procedure for microelectrodes. The completed arrays of microelectrodes are placed in a one-liter beaker and the tips were protected by clamping an aneurysm clip on the cable. They were then sonicated at low power in 95% ETOH for ten minutes followed by 10 minutes in Liquinex 1% solution. This was followed by rinsing 3 times by sonicating in 10 megohm deionized water, while exchanging the water after each rinse. Then, the container and array were air dried in the clean room. Next, the container and array were placed in a plastic sterilization bag together with an ethylene oxide indicator and sealed. This bag was placed into a second inner bag and sealed. The 2 inner bags were then placed into a third bag together with an ethylene oxide (EtO) ampule and sealed. The ampule was then broken and sterilization then carried out in a designated fume hood.

Recently, we have installed a Hepa filter in our surgical suite as an added precaution.

Surgical Procedure. Aseptic technique is used during the surgical implantation of the microelectrode arrays. Young adult cats of either sex are anesthetized initially with Ketamine with transition to a mixture of nitrous oxide, oxygen and Halothane. The surgical procedure is carried out with the animal's head in a stereotaxic apparatus. The scalp and temporalis muscle are reflected and, using a Hall bone drill, a craniectomy is made over the left frontal cortex extending into the frontal sinus. The frontal air sinus is partly filled with bone cement.

Prior to implanting the intracortical microelectrode array, a monopolar recording electrode, and its accompanying reference electrode, are implanted in the cat's pyramidal (corticospinal) tract in order to record neuronal activity evoked by the stimulating microelectrodes. The recording electrode is fabricated from 0.25 mm Teflon-insulated stainless steel wire. The exposed area at the tip of the stainless steel electrode wire is approximately 0.1 mm^2. In preparation for implantation, the wire is

mounted in a sleeve-type cannula device, which is mounted in a stereotaxic assembly. A small burr hole is cut in the calvarium over the cerebellum, and a small incision is made in the dura. A stimulating macroelectrode (approximately 0.5 mm in diameter) is placed against the dura over the pre- or postcruciate gyrus. This macroelectrode can support a large stimulus current (1 to 2 mA, 150 μsec/ph, at 20 Hz) which excites many corticospinal neurons and thus produces a large compound action potential that can be used to guide the recording electrode into the pyramidal tract. When the tip of the electrode is in the pyramidal tract, the inner introducer is retracted, and the recording and reference electrodes are sealed to the skull with bone cement.

In preparation for implanting the microelectrode array into the sensorimotor cortex, the percutaneous connector is mounted to the skull with stainless steel screws and methacrylate bone cement. A flap is made in the dura over the postcruciate cortex, and the array of microelectrodes is inserted into the cortex with the aid of an axial introducer mounted on the stereotaxic frame. Prior to animal IC-176, the array was inserted slowly, at approximately 0.25 mm/sec. In these animals, the arrays were implanted at a velocity of approximately 1m/sec. In animal IC-199 and later, the dura was drawn back over the array, using pre-installed 7-0 sutures in the dural flap. The dura was not closed completely, but the entire array was pressed against the pia. The openings between the dural edges were covered with a sheet of fascia resected from the temporalis muscle. The cortex and fascia patch were covered with autologous fat or fascia, to fill as much of the space as possible. The ground electrode was placed medial to the craniectomy defect. The muscle and skin were then closed in layers.

Stimulation Protocols. The test stimulation protocols were conducted at least 80 days after the implant surgery. During the stimulation, the cats were lightly anesthetized with Propofol, and the stimulation and recording was conducted through a percutaneous connector fixed to the skull. We have determined that the electrical excitability of the corticospinal neurons is not altered by light Propofol anesthesia, and the cats are much easier to manage when lightly anesthetized than when awake and wearing the tethered backpack. The microelectrodes were pulsed sequentially (interleaved stimulation). The stimulus was charge-balanced, controlled current biphasic pulse pairs, 150 μs/phase (cathodic phase first). The activated iridium

microelectrodes were biased at +400 mV with respect to the implanted Ag/AgCl reference electrode. The stimulation protocols are presented in Table I.

Histologic Methods. Thirty to 90 minutes after the end of the stimulation regimen, the cats were deeply anesthetized with pentobarbital, heparinized, and one liter of 0.9% NaCl perfused through the ascending aorta using a peristaltic pump (Model #2520-55, Cole Palmer Instrument Co.) at 120 mm Hg. This prewash was followed by one liter of ½ strength Karnovsky's fixative (2.5% glutaraldehyde, 2% paraformaldehyde and 0.1 M sodium cacodylate buffer).

The brain was left in fixative overnight and the following day, the array of microelectrodes was removed from the cerebral cortex after careful dissection of the overlying connective tissue and dura. The block of tissue containing the array tracks was resected, embedded in paraffin, sectioned serially in the horizontal plane (perpendicular to the shafts of the stimulating microelectrode) at a thickness of 8 μm. Alternate slides (5-6 sections) were stained with cresyl violet (Nissl stain) and H&E. Occasional sections near the electrode tips were stained with an antibody to glial fibrillary acidic protein (GFAP).

RESULTS

Physiologic results.

The neuronal activity evoked in the ipsilateral pyramidal (corticospinal) tract by each of the intracortical microelectrodes was recorded via the electrode implanted chronically electrode in the tract. Due to the sparseness of the corticospinal projection from the feline sensorimotor cortex, it is necessary to summate (average) the response to 2048 successive stimulus pulses, in order to obtain an acceptable signal-to-noise ratio. When an intracortical stimulating microelectrode did evoke a response, it had the characteristics of having been generated by a single corticospinal neuron. The "unit-like" responses exhibit "all-or-none" behavior and are of short duration, as illustrated in Fig. 1. The unit-like response is circled. This ability of our pyramidal tract electrode to record the responses from what appear to be single corticospinal neurons can be

TABLE I
STIMULUS PARAMETERS

IC #	# ELECTRODES PULSED (16 Electrode Arrays)	UNPULSED ELECTRODES (16 Electrode Arrays)	PULSE RATE (Hz)	PULSE AMPLITUDE (μA)	PULSE DURATION (μA)	CHARGE/ ph. (nC)	CHARGE DENSITY (μC/cm²)	DUTY CYCLE (Sec)	STIM. DURATION	POST-STIM. TIME
199*	1-7,9-11,13-16	8,12	100	20	400	8	400	0.25 on; 1.0 off	7 hrs/day for 3 days	90 min
195*	3,6,7,9,10,14,15,16	none	100	20	400	8	400	1.0 on; 1.0 off	7 hrs/day for 3 days	30 min
195*	1,2,4,5,8,11-13	none	100	20	400	8	400	con-tinuous	7 hrs/day for 3 days	30 min
191**	1,8,10,12	2,4-7, 9,11,13,15,16	50	50	400	20	1,000	con-tinuous	7 hrs.	45 min.
191**	3,14	2,4-7, 9,11,13,15,16	50	100	400	40	2,000	con-tinuous	7 hrs.	45 min.

*Animals used for evaluation of stimulation protocols used to produce phosphenes in human visual cortex (Schmidt et al., 1996); (the first animal was the Schmidt protocol; others were variations with increasing duty cycles.)

**Electrodes pulsed for determination of neuronal damage threshold.

attributed to the sparseness of the corticospinal projection, and the fact that any corticospinal neurons that are excited by the intracortical microelectrode must pass very close to the recording electrodes, in order for their action potentials to be detected. The abscissa is the latency after the start of the 150 μs/phase biphasic stimulus pulse. The number near the right edge of each trace is the amplitude of the stimulus pulse. Since the unit-like response was not evoked by a stimulus pulse of 6 μA and was present at 8 μA, its threshold was considered to be 8 μA.

Fig. 2 shows 27 unit-like responses evoked from the 16 microelectrodes implanted in cat IC-200. The latency of these unit-like responses after the stimulus pulse was less than 2 ms, so we presume that they represent corticospinal neurons that were activated directly rather than transsynaptically, by the intracortical microstimulation. The responses were evoked as the microelectrodes were pulsed individually with cathodic-first (CF) pulses, 150 μs/phase in duration. The cat was anesthetized lightly with propofol. Each circle represents one unit-like response. The abscissa is the initial threshold of the unit-like response. This was a "negative control" experiment; during the subsequent 7 hours, the anodic bias was applied to the microelectrodes but no stimulus was delivered. The ordinate is the threshold of the response after 7 hours. Some clusters are labeled to indicate that there is more than one unit at that location (for clarity, the members comprising these clusters are graphed slightly displaced from their actual values). The diagonal line represents the condition in which initial threshold = threshold after 7 hours (the condition in which the unit's electrical threshold was unchanged). The broken lines represent a threshold change of + or - I stimulus level (essentially, the resolution of the technique). During the 7 hours, none of the thresholds increased by more than 1 level, and the thresholds of 2 of the responses actually decreased.

Fig. 3A shows the effect of 7 hours of pulsing of 14 of the 16 microelectrodes in cat ic199. The stimulus parameters (20 μA CF pulses, 400 μs/phase, 8 nC/phase, 100 Hz, delivered with a duty cycle of 0.25 sec on, 1 sec off) produced stable phosphenes when delivered through microelectrodes implanted in the visual cortex of a human volunteer (Schmidt et al, 1996). The 17 unit-like responses were recorded from the 14 pulsed electrodes. However, the responses were evoked before and after the 7 hours of stimulation using a pulse duration of 150 μs/phase, rather than 400 μs, to avoid

problems with the stimulus artifact when recording the responses of units with very short post-stimulus latencies. Most of the thresholds were stable during the 7 hours of stimulation, and only 2 were slightly and 1 moderately elevated above the +1 level. Figure 3B shows the effect of the same protocol on the thresholds of 22 unit-like responses recorded from another cat (IC-201) which has not yet been sacrificed. In this animal, all 16 electrodes were pulsed. Again, the stimulation produced only a small elevation in the threshold of 4 of the 22 responses. These results are consistent with the absence of detectable histologic changes in cat IC-199, in which the 14 of the 16 intracortical microelectrodes were pulsed with these parameters for 7 hours per day on 3 successive days, prior to being sacrificed for histologic evaluation (Figs. 4A,B,C). The minimal effect of the stimulation on neuronal excitability can be attributed to the use of a short duty cycle. When the stimulation is continuous for 7 hours, there is marked elevation of neuronal thresholds, even when the pulsed rate and charge per phase are less (e.g., 50 Hz, 4 nC/phase) as illustrated in Fig. 3C.

Histologic results.

Table I presents the stimulus parameters used for the two studies. Electrodes from Animals IC-199 and IC-195 were pulsed with the parameters used to produce phosphenes in the human visual cortex (Schmidt, et al., 1996), or variants of this protocol in which the stimulus duty cycle was greater (one second on, one second off or else continuous stimulation). There was no neuronal injury at the pulsed or unpulsed tip sites with any of the 3 sets of parameters. Fig. 4A shows an unpulsed electrode tip site (#12, IC-199). The tip site of the electrode is surrounded by a small amount of gliosis, with many normal-appearing neurons nearby. Figs. 4B and 4C are pulsed tip sites #4 and #5 (IC-199) which appear essentially the same as the unpulsed site, i.e., a small amount of gliosis around the tip and normal-appearing neurons. There is some aggregation of lymphocytes around the pulsed tip sites, and some cuffing of lymphocytes around nearby small blood vessels. Even with a continuous duty cycle, there were no neuronal changes (Fig. 5A, #8, IC-195 and 5B, #9, IC-195). Note that at one of the tip sites (Fig. 5A), gliosis was present, but gliosis was absent in Fig. 5B (as we have previously reported (QPR #6), gliosis occurs randomly at tip sites and along the electrode shafts).

In the second study designed to determine the neuronal damage threshold, two levels of intense stimulation were employed. Fig. 6A shows an unpulsed electrode site from animal IC-191, surrounded by normal-appearing neurons. The lower intensity stimulation (20 nC/ph and 1,000 μC/ph, continuous stimulation for 7 hours at 50 Hz), showed a suggestion of neuronal injury. This was in the form of a few neurons with disrupted plasma membranes and prominent dendritic profiles. The profiles of these neurons close to the electrode tip appeared "smudgy," while other neurons subjected to this protocol and close to the tip appeared normal (Figs. 6B and C).

When the stimulus intensity was increased to 2,000 μC/cm², 40 nC/ph, neuronal changes were similar to those induced by the lower intensity stimulus, but a significantly greater number of neurons were involved (Figs. 7A and B).

DISCUSSION

The lack of neuronal injury by microstimulation in the cerebral cortex using parameters identical to, or exceeding in intensity, those used by Schmidt et al., 1996, to produce phosphenes in the human, is encouraging. These findings are important for any future FES application of microstimulation in the brain. When the duty cycle runs low (0.25 sec on, 1 sec off), there also was only minimal depression of neuronal excitability, whereas, with continuous duty cycle the depression of neuronal excitability was significantly greater.

Our determinations of neural damage threshold in the second series (intense stimulation protocols), need to be confirmed and extended by evaluation with transmission electron microscopy; however, they do suggest that the neuronal damage threshold for closely-spaced microelectrodes is between 20 nC (1,000 μC/cm) and 40 nC (2,000 μC/cm²). It should be noted that the morphologic changes observed in these studies are quite distinct from those observed when a greater volume of tissue is activated, and when many neurons are damaged by intense stimulation from the brain surface. In those studies, large numbers of neurons underwent marked shrinkage (stellate profiles), hyperchromicity and were accompanied by perineuronal haloes (Agnew et al., 1993).

The present study is the first instance in which we have observed neuronal injury in the cat's cortex following microstimulation. Further studies are also needed to

determine the role of mass action, e.g., do multiple adjacent electrodes pulsed at a given intensity induce a proportionately greater amount of neuronal damage than when single microelectrodes are pulsed with the same parameters? We also need to employ immunohistochemical markers to better define more subtle changes due to neuronal hyperactivity and also to correlate this with changes in neuronal excitability as reported in this and other studies.

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2. Liu, X., McCreery, D.B., Carter, R.R., Bullara, L.A., Yuen, T.G.H. and Agnew, W.F.: Stability of the interface between neural tissue and chronically-implanted intracortical microelectrodes. IEEE Trans. Rehab. Eng., 7(3):315-326, 1999.
3. Schmidt, E.M., Bak, M.J., Hambrecht, F.T., Kufta, C.V., O'Rourke, D.K. and Vallabhanath, P.: Feasibility of a visual prosthesis for the blind based on intracortical microstimulation of the visual cortex. Brain, 119:507-522, 1996.

WORK NEXT QUARTER

We will conduct experiments to more closely define the neuronal damage threshold, as well as mechanisms of electrically-induced neural damage, using high intensity stimulations with the use of both transmission electron microscopy and immunocytohistochemical applications.

cat ic199 July 11, 2000. Before stimulation.

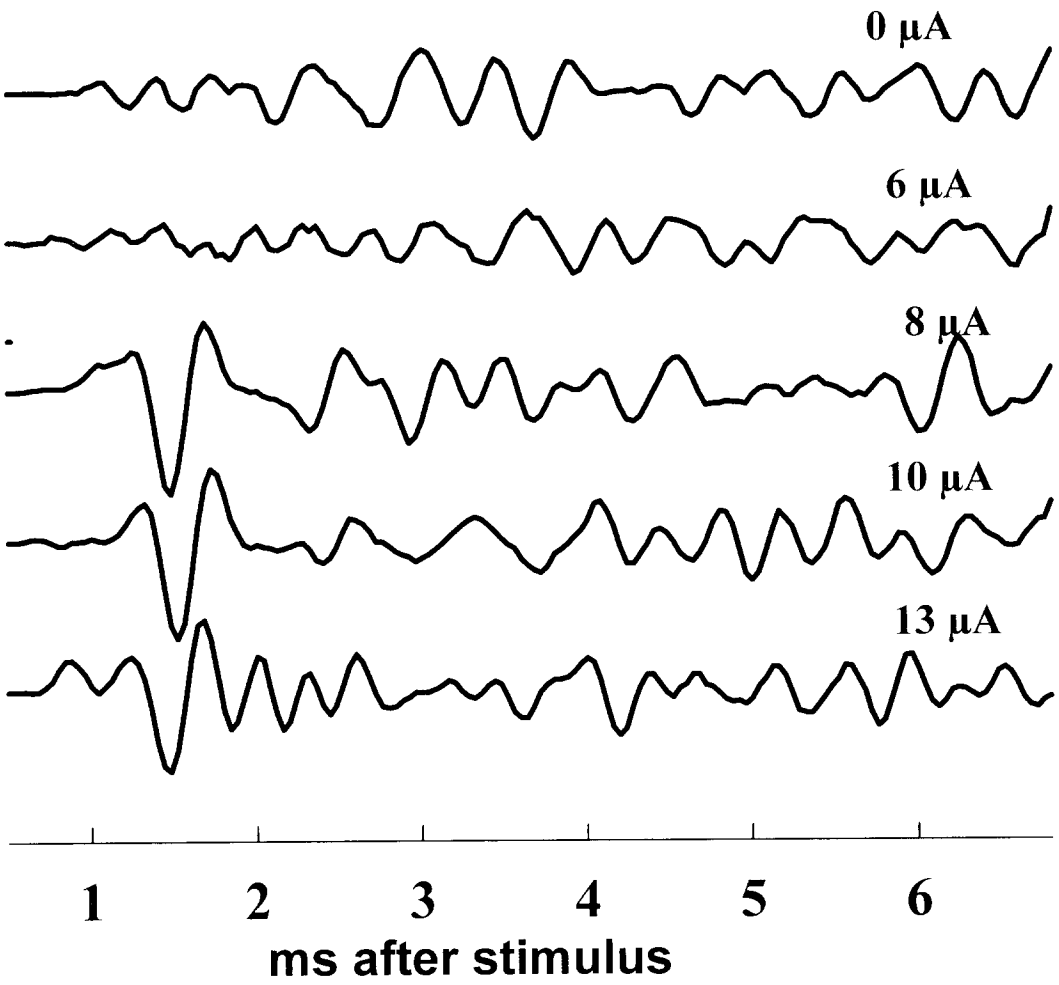

Figure 1

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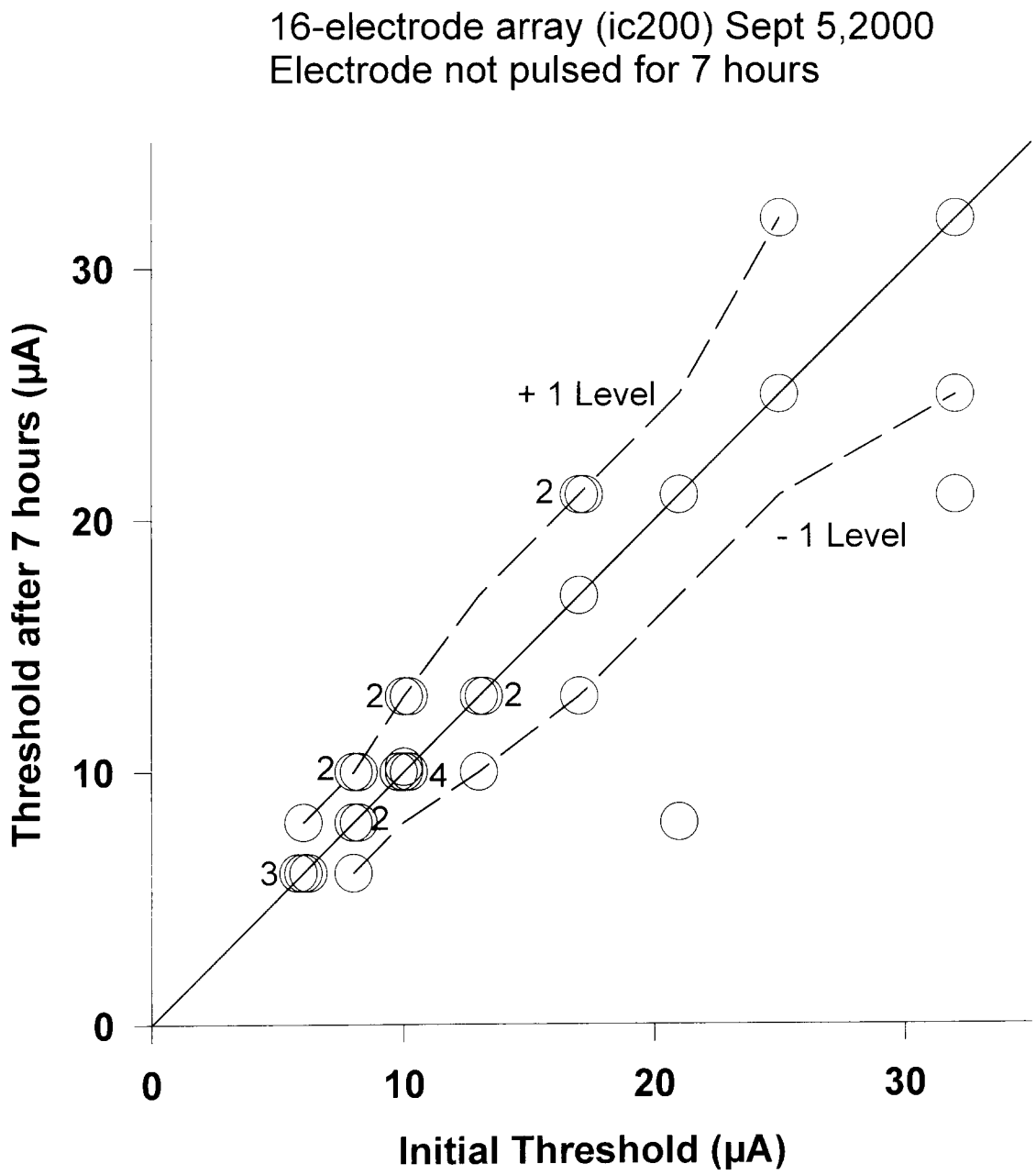

h:/spw/ic/th_200b.spw

Figure 2

16-electrode array (ic199) Sept 18-20,2000 (101 days after implantation)
14 electrodes pulsed simultaneously

20 µA, 400 µs/phase, (100 Hz, 0.25 sec on, 1 sec off for 7 hours)
data from first day (17 unit-like responses)

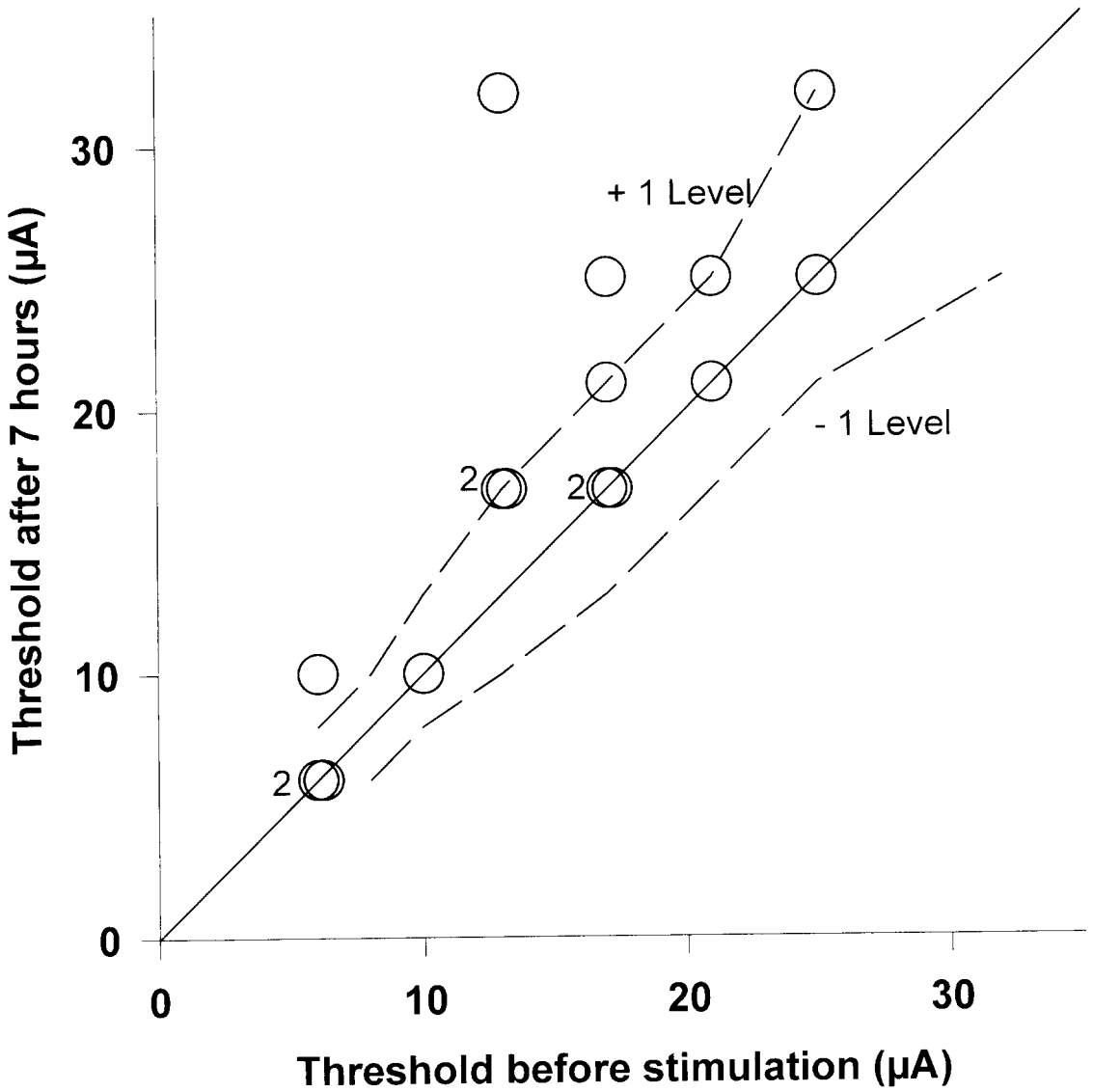

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Figure 3A

16-electrode array (ic201) Sept 26, 2000 (68 days after implantation)
16 electrodes pulsed simultaneously

20 µA, 400 µs/phase, (100 Hz, 0.25 sec on, 1 sec off for 7 hours)
(22 unit-like responses)

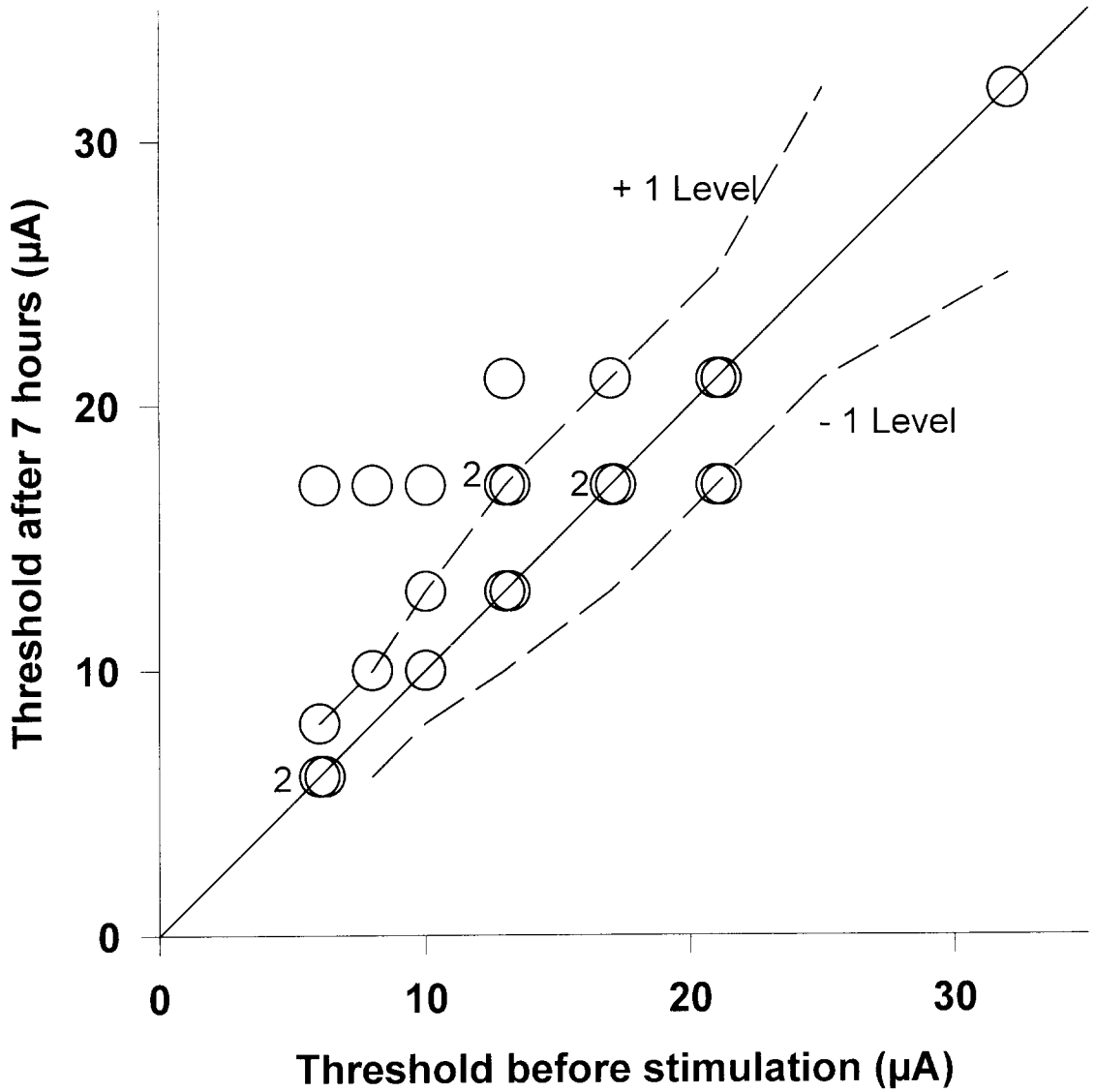

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Figure 3B

16-electrode array
15 of 16 electrode pulsed in interleaved mode
16 unit-like responses
26.5 µA (4 nC/phase), 50 Hz, continuous

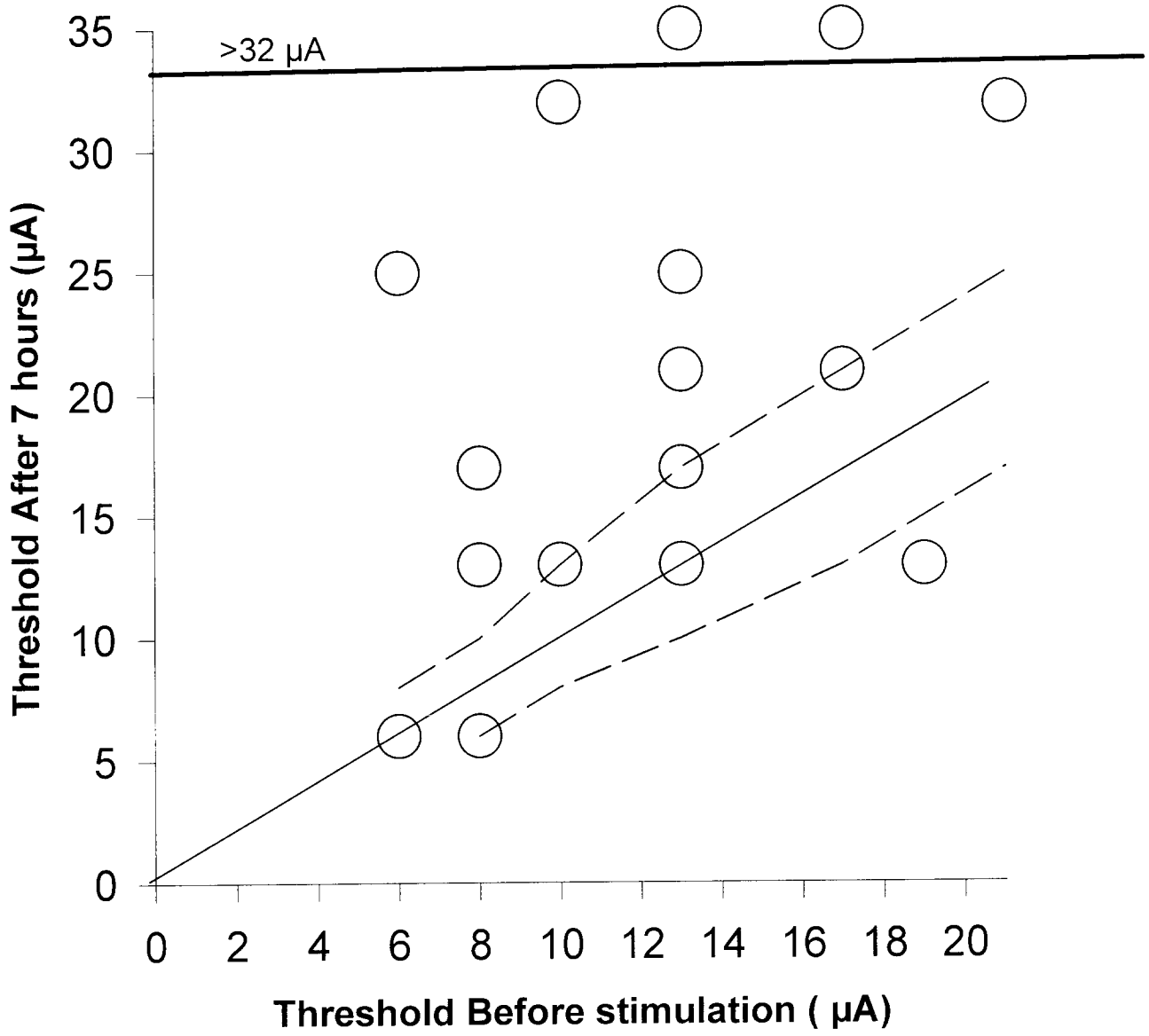

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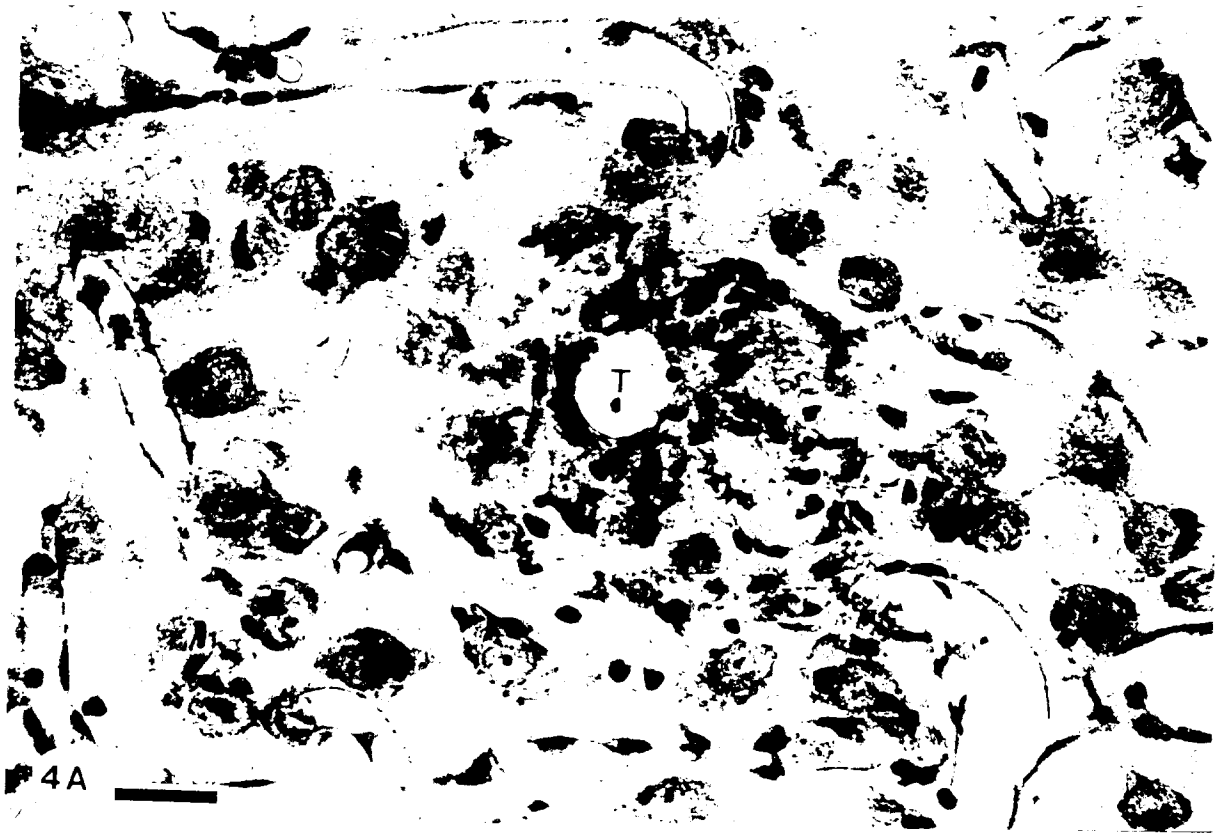
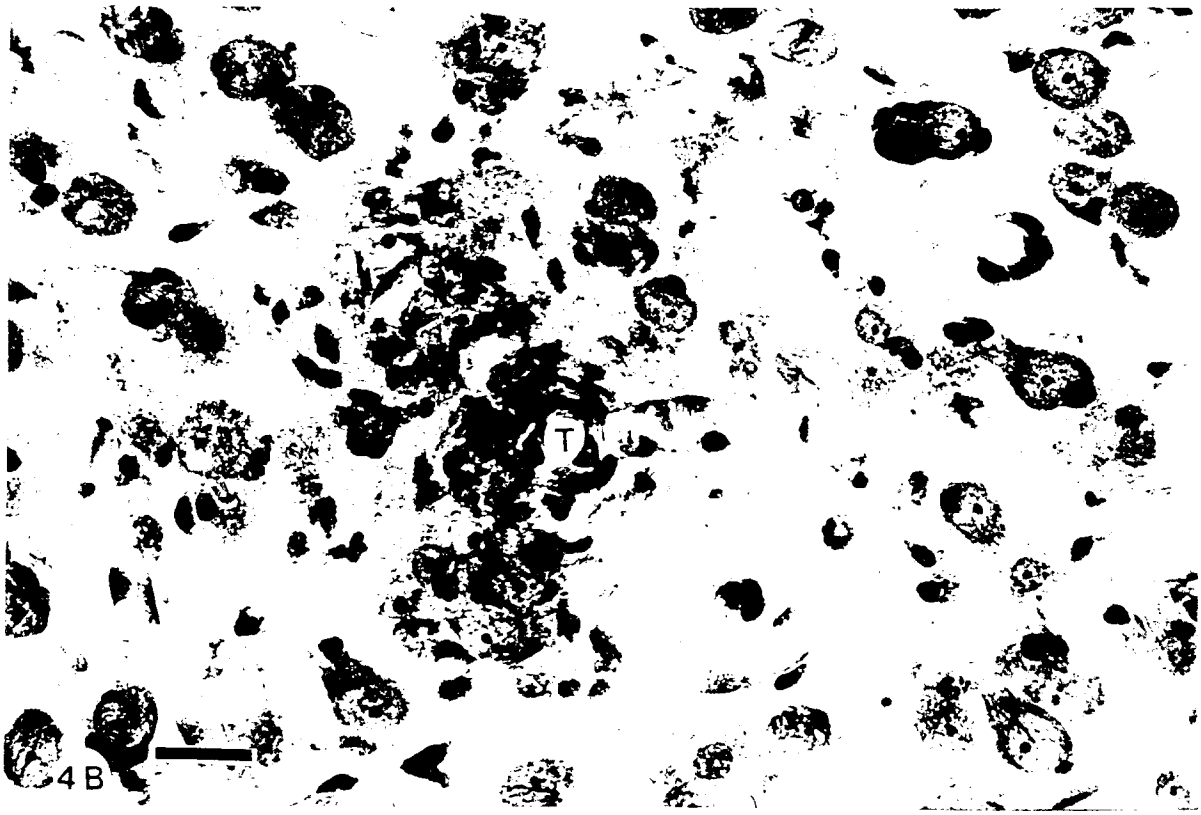
Figure 3C

FIGS. 4A - 7B

Fig. 4A. IC-199. Unpulsed tip site of electrode no. 12 at 101 days after implantation. A few gliotic cells surround the tip site (T) including occasional lymphocytes (arrows). Neurons throughout the field appear normal, including some within 60 µm of the tip. Nissl stain. Bar = 30 µm. This and all succeeding micrographs are from paraffin sections cut at 8 µm.


T
4A

Figs. 4B and 4C. Pulsed tip sites nos. 4 and 5. Both were pulsed with a pulse amplitude of 20 μA and a charge per phase of 8 nC at a charge density of 400 μC/cm^2 and a duty cycle of 0.25 sec on and 1 sec off. Each micrograph shows an electrode tip (T) surrounded by slight gliosis and numerous normal-appearing neurons. Nissl stain. Each Bar = 30 μm.


T
4 B

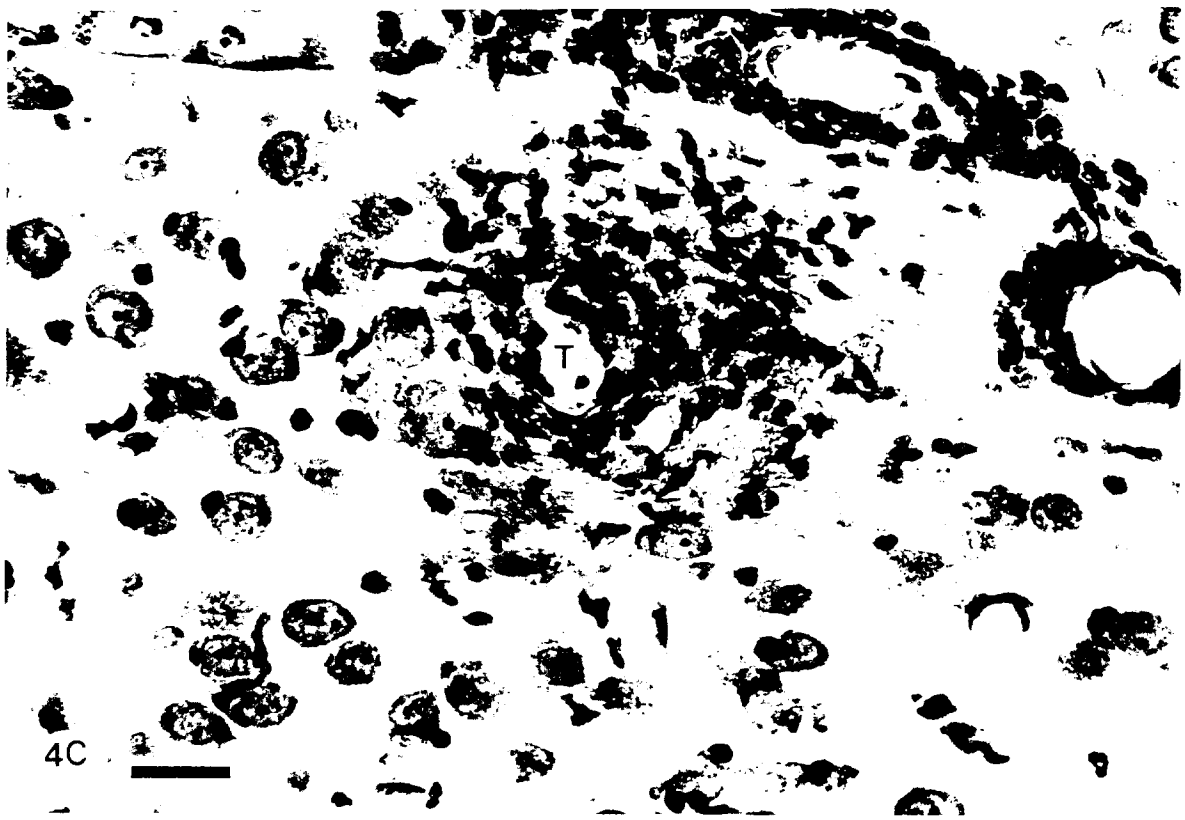
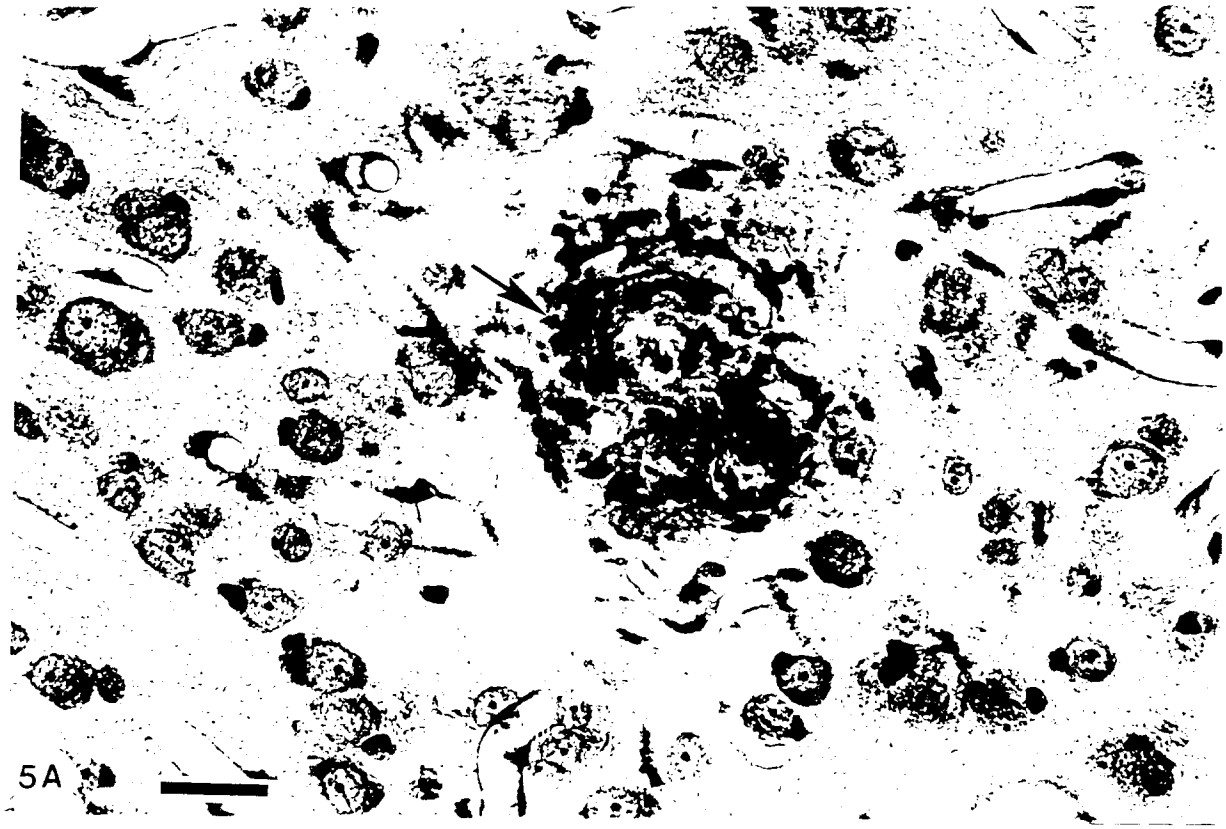

T
4C

Fig. 5A. IC-195. Tip site of electrode no. 8 pulsed continuously for 7 hr. × 3 days with a pulse amplitude of 20 μA, a charge density of 400 μC/cm², a charge per phase of 8 nC and sacrificed 30 minutes after stimulation. The duty cycle was continuous. A slight gliosis surrounds the tip site (arrow). All neurons appear normal. Nissl stain. Bar = 30 μm.

Fig. 5B. Tip site of electrode no. 9 (arrow) located adjacent to electrode no. 8 in the previous micrograph and pulsed with parameters identical to those for electrode no. 8. Gliosis is not present and there is no neuronal damage. Nissl stain. Bar = 30 μm.


5A

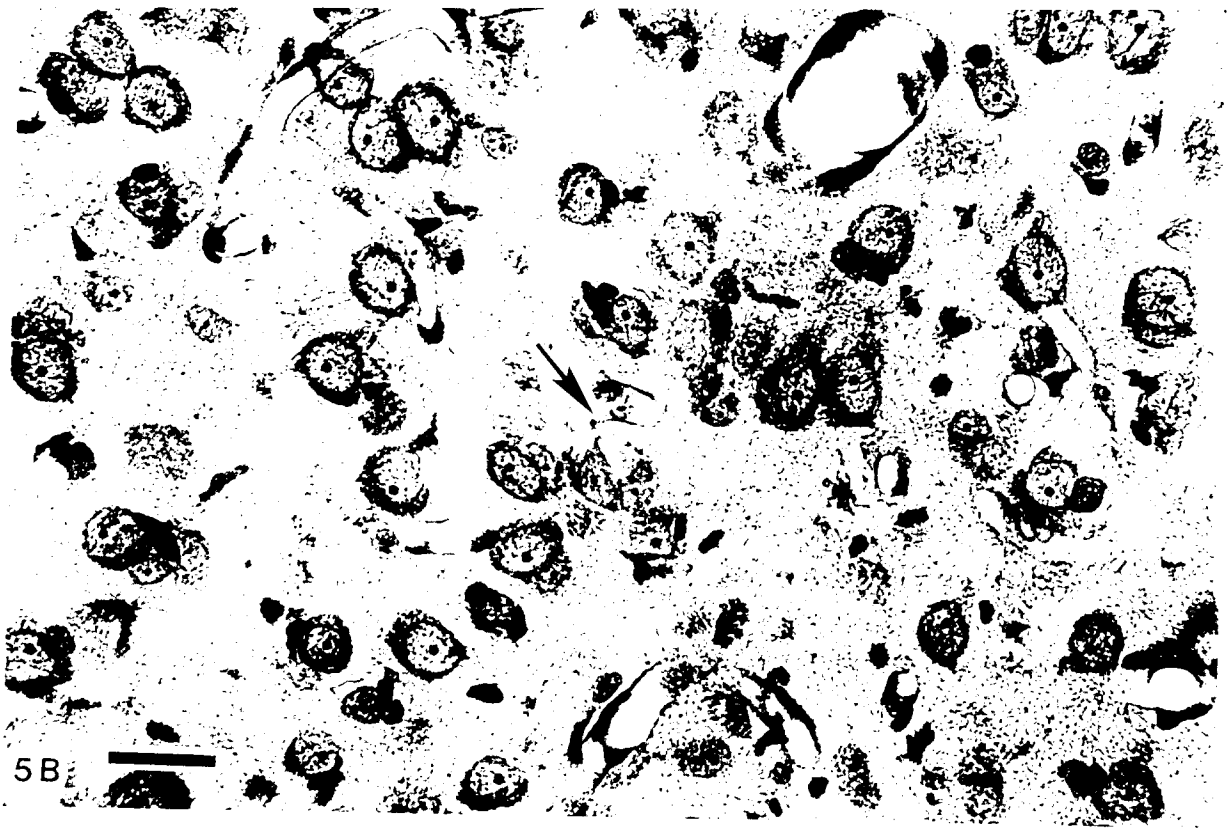

5B

Fig. 6A. This and all succeeding micrographs are from IC-191. Unpulsed tip site (T) of electrode no. 16, 330 days following implantation. Occasional glial cells are present around the tip site. Neurons at all distances form the tip site appear normal. H&E stain. Bar = 30 μm.

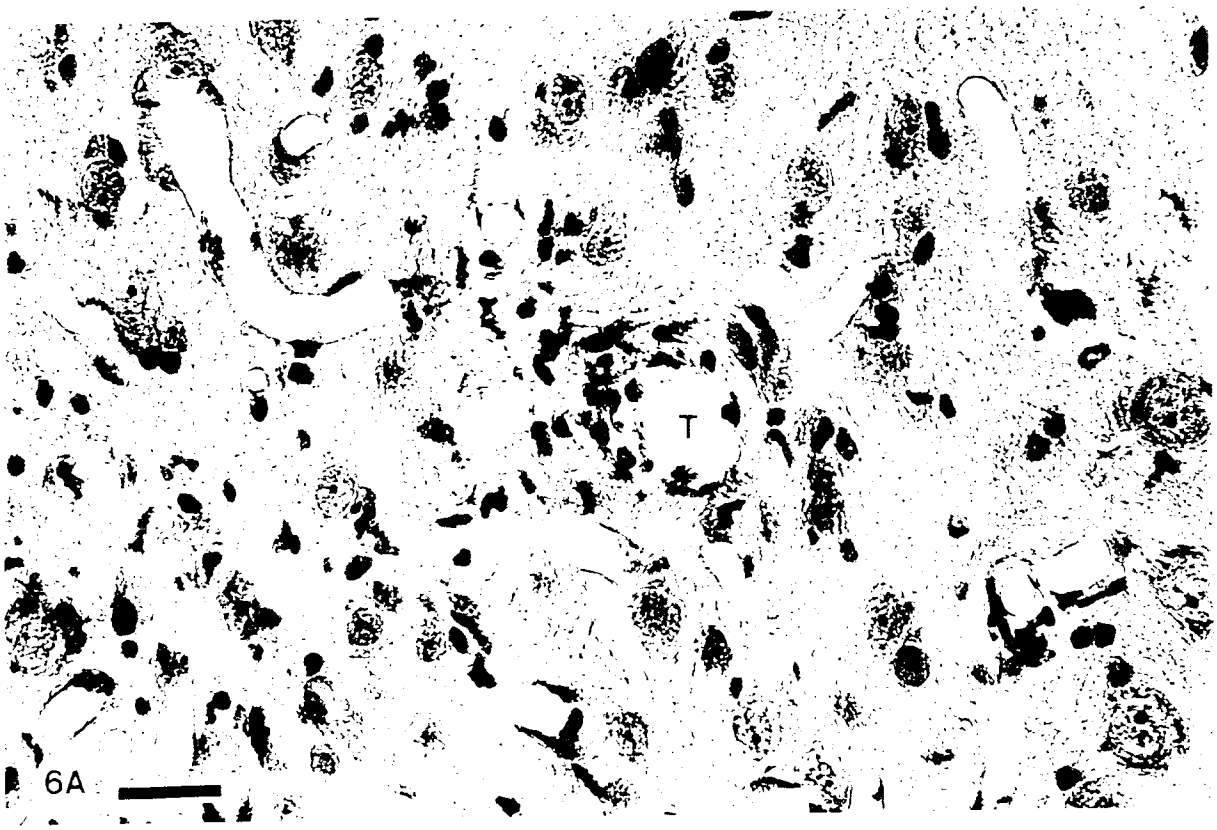
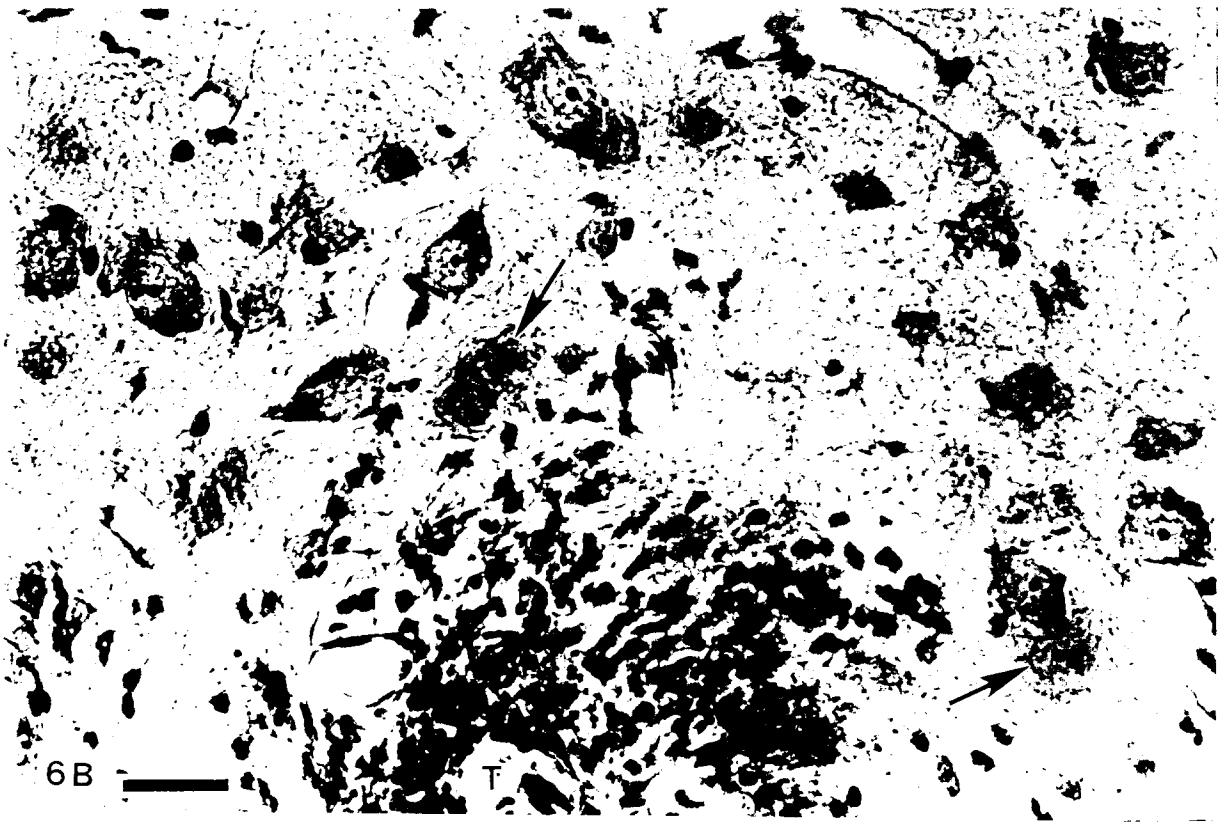

T
6A

Fig. 6B. Oil-immersion micrograph of tip site no. 12 (T) pulsed continuously for 7 hours with a pulse amplitude of 50 μA and a charge per phase of 20 nC. The charge density was 1,000 μC/cm² and the animal was sacrificed 45 minutes following stimulation. A few neurons near the tip appear to be fragmenting (arrows) while most others appear normal. Nissl stain. Bar = 30 μm.

Fig. 6C. Oil immersion micrograph of the region to the right side of Fig. 6B showing marked disintegration of one neuron (arrow) while others appear more normal. Nissl stain. Bar = 15 μm.


6B
T

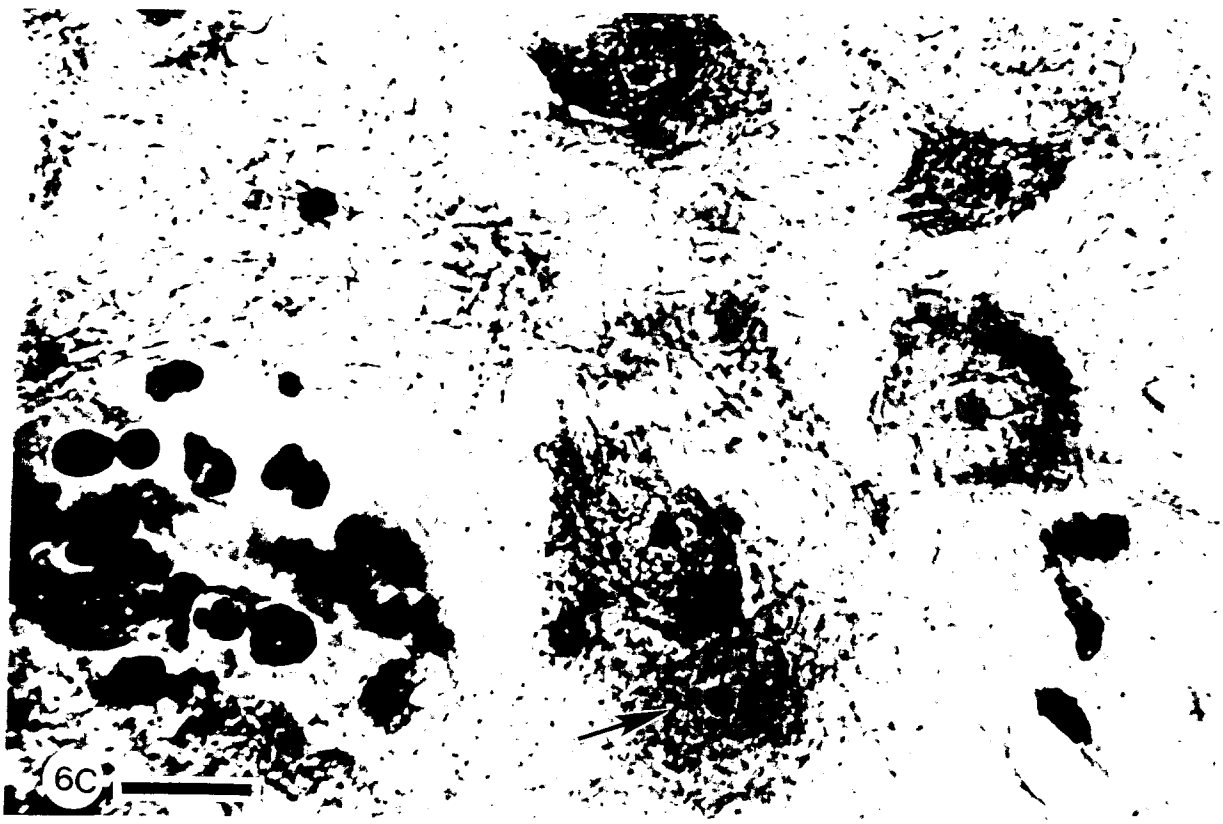
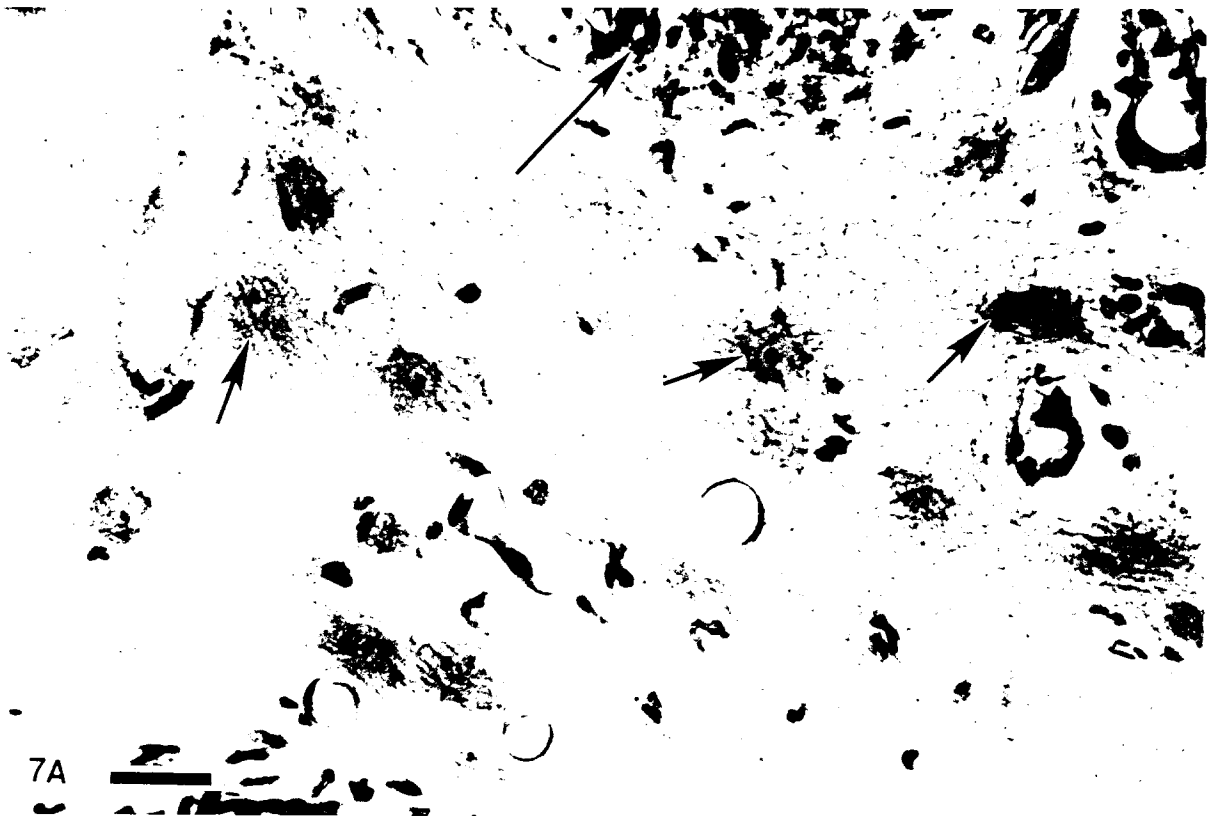
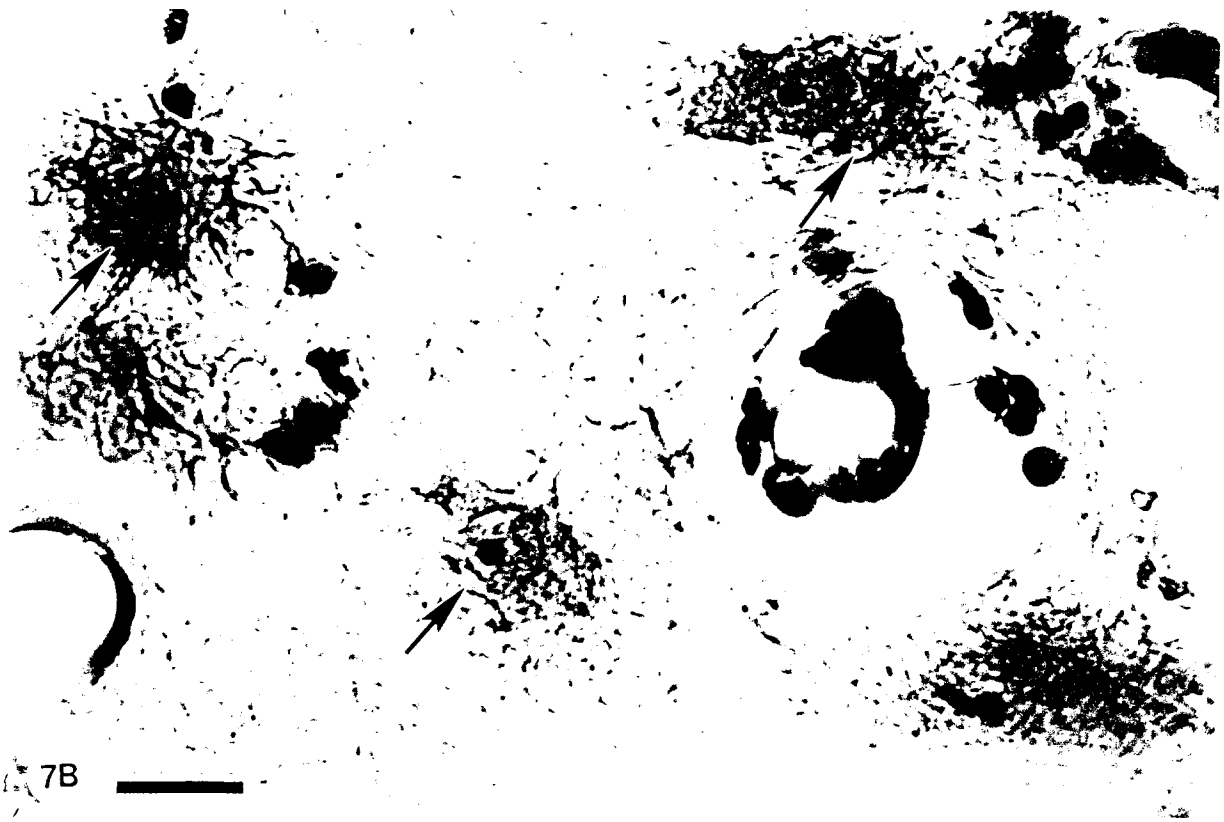

6C

Fig. 7A & 7B. Low magnification and oil-immersion micrographs (respectively) of tip site no. 3 surrounded by moderate gliosis (longer arrow). Stimulation parameters included pulsing for 7 hours continuously at a pulse amplitude of 100 μA and a charge/phase of 40 nC. The geometric charge density was 2,000 μC/cm². Several neurons (arrows) near the tip site show damage in the form of disturbance of the plasma membrane and numerous abnormally prominent dendrites. Nissl stain. Bars = 30 and 15 μm, respectively.



7A



7B